Ceruloplasmin concentration in dogs with multicentric lymphoma undergoing chemotherapy

Concentração de ceruplasmina em cães com linfoma multicêntrico submetidos à quimioterapia

Sílvia Regina Ricci LUCAS[1]; Alexandre MERLO[1]; Regina Mieko Sakata MIRANDOLA[1]; Thais Paione GASPARIN[1]

[1]School of Veterinary Medicine and Animal Science at the University of São Paulo, Brazil

Abstract

Ceruloplasmin (Cp) is a positive acute phase protein, responsible for the transport of copper and protection of cells and tissue against oxidant compounds. The aim of this study was to evaluate Cp concentrations at diagnosis and during chemotherapy in dogs with multicentric lymphoma (ML). Cp was measured using orto dianisine technique, in two groups of dogs: ten healthy dogs (control) and 13 dogs with ML. All dogs were submitted to chemotherapy. Dogs with signs of concurrent disease or that have been previously treated with prednisone were excluded from the study. Cp measurement was done before treatment and once a week, during the first month of chemotherapy, and each 3-week intervals until the relapse for dogs with ML, and until the 16th week in control dogs. ANOVA test followed by multiple Tukey's tests were used to compare the groups. There was no difference between the mean of Cp concentration in dogs with ML at the diagnosis when compared to healthy dogs ($p > .05$). Levels of Cp decreased significantly at 4th week when compared to the first week, but Cp increase was not observed at the relapse. At all other times during the treatment, Cp concentrations for dogs with lymphoma were not significantly different from controls submitted to chemotherapy. As conclusion, Cp levels in ML at diagnosis were similar to healthy dogs, decreased when lymphoma remission was achieved and there was no change at the relapse.

Keywords: Ceruloplasmin. Lymphoma. Dogs. Acute phase protein.

Resumo

Ceruloplasmina (Cp) é uma proteína positiva de fase aguda, responsável pelo transporte de cobre e proteção das células e tecidos contra compostos oxidantes. O objetivo deste estudo foi avaliar as concentrações de Cp no momento do diagnóstico e durante a quimioterapia em cães com linfoma multicêntrico (LM). Cp foi mensurada utilizando-se a técnica da orto dianisina em dois grupos de cães: dez cães sadios (controle) e 13 cães com LM. Todos os cães foram submetidosàa quimioterapia. Cães com sintomas de doenças intercorrentes ou que haviam sido previamente tratados com prednisona foram excluídos do estudo. Cp foi mensurada antes do início do tratamento e uma vez por semana durante o primeiro mês de quimioterapia e depois a intervalos de três semanas até a recidiva.para cães com LM, e até 16 semanas nos cães do grupo controle. ANOVA seguida pelo teste de Tukey foi usada para comparar os grupos. Não houve diferença entre a media das concentrações de Cp em cães com LM ao diagnóstico quando comparados aos cães sadios ($p > 0.05$). Os níveis de Cp diminuíram significativamente na quarta semana de tratamento comparado ao momento do diagnóstico. Aumentos nas concentrações de Cp não foram observados na recidiva. Durante o tratamento, as concentrações de Cp em cães com linfoma não diferiram daquelas observadas nos animais do grupo controle submetidos à quimioterapia. Concluiu-se que os níveis de Cp nos cães com LM ao diagnóstico foram similares aos cães sadios, diminuiu quando se obteve a remissão e não se alterou na recidiva da doença.

Palavras-chave: Ceruloplasmina. Linfoma. Cães. Proteínas de fase aguda.

Introduction

The acute phase response is considered a part of innate host defense system, which is responsible for the survival of the host during the critical early stages of attack[1]. Inflammatory, infectious, immunologic, neoplastic conditions and other injuries, such as surgery, can stimulate thse response, which includes changes in the concentrations of determined plasma proteins, called acute phase proteins (APPs). There are negative

Correspondence to:
Sílvia Regina Ricci LUCAS
Av. Prof. Dr. Orlando Marques de Paiva, 87, blocos 12/14
CEP 05508-270- São Paulo, SP, Brasil
E-mail: srrlucas@usp.br

Received: 13/04/2009
Approved: 05/08/2010

APPs, which decrease in concentration during the response (e.g albumin) and positives APPs, that increase during acute phase response (e.g ceruloplasmin)[2].

Positive APPs are synthesized mainly by hepatocytes under stimulation of inflammatory cytokines such as IL-1, IL-6 and TNF- α. and represent a very fast and nonspecific response that occurs in many cases before clinical signs[3]. Production and response of APsS varies depending on the species and biological characteristics of the protein. Ceruloplasmin (Cp) is a positive alfa 2 glycoprotein in dogs[4] that is responsible for the transport of copper and protection of cells and tissue against oxidant compounds, generated by phagocytes in the course of clearing microorganisms of tissue debris[2].

Multicentric lymphoma (ML) is a common neoplasia in dogs, and systemic chemotherapy is indicated for long-term[5]. Despite the fact that ML is more sensitive to chemotherapy than other tumors, treatment is generally palliative and the objective is to achieve remission for extensive periods[6,7]. Several protocols utilizing combinations of chemotherapeutic agents such as prednisone, vincristine, cyclophosphamide doxorubicin and metotrexate are used and there is no consensus regarding the best treatment considering efficacy, remission and survival times then, relapse is common during chemotherapy and usually requires drug regimen modifications[5,8] then, chemotherapy changes before clinical relapse could help to control cancer progression.

In humans, some APPs has been used as a prognostic factor for lymphoid neoplasias, as well as for monitoring cancer remission and relapse[1,9,10,11]. In dogs, some acute phase proteins were studied for monitoring diseases and cancer such as haptoglobin, C reactive protein and serum amyloid A[3,12,13] with variable results, but there are no studies about the use of Cp for monitoring canine lymphoma. The purpose of this study was to evaluate the levels of Cp in dogs with multicentric lymphoma undergoing chemotherapy and if there are changes during the treatment.

Material and Method

Ten healthy dogs anen13 dogs with ML were included in the study. The experimental protocol was approved by the Bioethics Committee of the University of São Paulo, School of Veterinary Medicine and Animal Science, in São Paulo, Brazil.

For the ten healthy dogs, results of clinical examination, CBC, serum biochemical profile (urea nitrogen, alanine aminotransferase and alkaline phosphatase activities, total protein, albumin, calcium and phosphorus) and urinalysis were unremarkable. Animals with signs of disease or any abnormality in the tests performed were excluded from the study. These animals (university-owned animals) were included in the Control group (n = 10) and submitted to chemotherapy for 16 weeks, to determine the effects of chemotherapy on serum Cp concentrations.

Thirteen dogs, all private-owned patients with multicentric lymphoma, diagnosed on the basis of cytologic examination of a fine-needle aspiration of an enlarged, peripheral lymph node were studied. Physical examination, CBC, serum biochemical profile, urinalysis, thoracic radiography and abdominal ultrasonography were performed in all dogs, and the dogs were classified as having stage IV or V multicentric lymphoma. Animals with signs of concurrent disease, localized infection, previous undergone chemotherapy or that were treated with prednisone were excluded from the study, as well as dogs presenting complications during treatment. Response was classified as complete remission (CR) (ie, disappearance of all solid tumors and clinical signs), partial remission (PR) (ie, ≥ 50% decrease in the size of tumor), or no response (ie, < 50% reduction in tumor size). Only animals with complete or partial remission were included in the study.

The dogs (control and lymphoma groups) were treated under CVP or VCMA protocol. CVP protocol consisted of cyclophosphamide (250 mg/m^2,

PO), vincristine (0,75 mg/m^2, IV) and prednisone (40 mg/m^2, q 24 h, PO), at week 1. Subsequently, the dogs received vincristine once a week for thre3 weeks and then at 3-week intervals; cyclophosphamide at 3-week intervals and prednisone (20 mg/m^2) on alternate days until relapse. VCMA protocol consisted of administration of vincristine consisted of administration of vincristine (0.75 mg/m^2, IV) during weeks 1 and 3; cyclophosphamide (250 mg/m^2, PO) during week 2, and administration of methotrexate (0.8 mg/kg [0.36 mg/lb], IM) during week 4. A single dose of l-asparaginase (10,000 U/m^2, SC) was given on day 1. This monthly schedule was maintained until relapse was detected. A CBC was performed before every treatment was administered, and chemotherapy was delayed if the neutrophil count was < 2,500 cells/μL.

In ten healthy dogs undergoing chemotherapy, blood samples were collected at week 1 (prior to treatment), 2, 3, 4, 7, 10, 13 and 16. In the dogs with lymphoma, the samples were collected before drug administration at weeks 1 (prior to treatment), 2, 3 and 4, at disease relapse, when the enlargement of the lymph nodes was detected. For all groups, serum was harvested and frozen at -70 ºC until assayed for Cp concentration.

Serum Cp concentration was determined by manual technique of Schonsinsky, Lehmann and Beeler[14], measuring the oxidative activity for this protein using orto dianisidine.

Data for Cp concentrations were analyzed by use of repeated-measures ANOVA following Tukey–Kramer Multiple Compariosns Test. Values o $p < 05$ were considered significant.

Results

Five CVP Control dogs ranged from to2 to for4 years of age and weighed between 12 and 23 kg, being for4 males (all intact) an 1one neutered female. Five VCMA Control dogs ranged from to2 to for4 years of age, and weighed between 12 and 19 kg, being thre3 males (neutered) and to2 neutered female.

Seven dogs with lymphoma assigned to CVP protocol ranged from to2 t10ten years old (mean 5,5 years) and weighed between 14 and 42 kg . Two were males (all intact) and fie5 were females (one neutered), being sx6 purebred and oe1 mixed-breed dogs. Six VCMA Lymphoma dogs ranged fro 3three t 8eight years old (mean 5,1 years old) and weighed between sx6 and 35 kg . Five were females (none neutered) and one was male, being all purebred.

Of the seven dogs with lymphoma assigned to CVP protocol, fie5 achieved a complete remission an 2two partial remission. Median of survival times was 257 days (SD = 105 days, range 75 to 390 days), mean time of remission was 104 days, (SD = 58 days, range 47 to 196 days) . In the dogs with lymphoma receiving VCMA protocol, all six dogs had a complete remission. Median of survival times was 297 days (SD = 131 days, range 109 to 498 days), mean time of remission was 167 days (SD = 85 days, range 56 to 267days).

Statistical analysis showed that there was no difference in relation to Cp levels, due to use of different treatment protocols. This is justified because Cp is not influenced by the use of corticoids. After this result, the groups were studied considering only the condition of control (healthy) and lymphoma.

For dogs with lymphoma, mean Cp at the first week (prior to treatment) was 21,67 U/L (range 10 to 31,87 U/L) and there was no difference when compared to healthy dogs, that presented Cp mean of 17,85 U/L (range 11,87 to 29,92) (p = 0,3868), table 1.

Considering the analysis of Cp concentrations in dogs with ML prior to treatment, on the 4th week (remission evaluation) and at the moment of relapse, there was a significative difference. The levels of Cp concentration decreased in the remission period when compared to the values observed at the diagnosis. There was no increase during the treatment, including the moment which the relapse was detected

Table 1 - Mean values and standard deviation of ceruloplasmin serum concentration (UI/L) during chemotherapeutic treatment in control and multicentric lymphoma (ML) groups, according weeks of treatment - São Paulo - 2008

WEEKS OF TREATMENT	GROUPS	
	Control (**n = 10**)	ML (**n = 13**)
1 (before treatment)	17.85 ± 5.45	21.67 ± 7.69
4 (remission evaluation)	19.87 ± 7.54	13.76 ± 5.79
Relapse	...	14.26 ± 5.93

($p > .05$). The Cp levels observed in the control group, undergoing chemotherapy at 4th week was higher when compared to ML group.

Discussion

The study of acute phase proteins and their association with lymphoma relapse arose from the needing to find an early marker for disease relapse, which occurs in most patients. Considering that the induction of a second remission is associated to high therapeutic failure[8], chemotherapy changes before relapse could help control cancer progression[13].

The acute phase response associated to canine lymphoma was described in some studies. Increased levels of C-reactive protein[3,12,15], serum amyloid A[13], α1-acid glycoprotein[16], and haptoglobin[3,15] had previously been detected in dogs with lymphoma before the chemotherapy, suggesting that the disease is a true stimulus for the hepatic synthesis of these proteins, similarly to what is observed in inflammatory conditions not related to cancer. Furthermore, levels of C-reactive protein[12], serum amyloid A[13], and α1-acid glycoprotein[16] gradually decreased in dogs that underwent chemotherapy and achieved a complete or partial remission, confirming the role of lymphoma itself on triggering the acute phase response. Ceruloplasmin is considered a positive acute phase protein, whose concentration usually increases upon stimulation by proinflammatory cytokines[2]. In this study, levels of ceruloplasmin in dogs with lymphoma prior to the chemotherapy were no higher than the levels observed in healthy dogs prior to the chemotherapy, what disagrees with the behavior of other positive acute phase proteins in canine lymphoma. Normal values of ceruloplasmin concentration in canine serum are 14,9 ± 2,6 UI/L, and, under inflammatory conditions, 20 UI/L[17]. Although ceruloplasmin is somewhat more specific than other acute phase proteins because it remains stable during steroid therapy, inflammation could lead to an increasing of only 50%, what clearly compromises its usefulness on accessing mild inflammatory conditions. In the only study in which ceruloplasmin was determined in the serum of canine patients with hematologic neoplasias (leukemia and lymphoma), there was no statistically significant difference between the levels observed in healthy and sick dogs[3]. These results coupled with the lack of induction of ceruloplasmin in our study suggest that it should not be used alone to evaluate the acute phase response in dogs with lymphoma. In this context, some authors postulate that ceruloplasmin is a moderate positive acute phase protein in dogs and cats owing to the low magnitude of response in inflammatory processes[18]. Indeed, ceruloplasmin should be determined in conjunction to a major acute phase protein like C-reactive protein and serum amyloid A in the dog or α1-acid glycoprotein and serum amyloid A in the cat[18].

Ceruloplasmin levels could be higher at the lymphoma relapse because of a new synthesis of acute phase proteins incited by volumetric expansion of the tumors (lymph nodes, spleen, and liver). However, levels of ceruloplasmin experienced a progressive de-

cline after starting the chemotherapy and kept above the pre-treatment values for both healthy and lymphoma dogs. In similar trials regarding the monitoring of dogs with lymphoma, abnormally elevated pre-chemotherapy values of C-reactive protein[12], serum amyloid A[13], and α1-acid glycoprotein [16] decreased following the treatment. Surprisingly, in this study ceruloplasmin declineat both in clinical remission and in relapse; its levels were lower than prior to the chemotherapy. This finding suggests chemotherapy influenced ceruloplasmin synthesis in dogs irrespectively of the presence of lymphoma. Taking into account ceruloplasmin contains eigt8 atoms of copper per molecule[2], a possible mechanism involving cupric metabolism could be involved. In a report, dogs with lymphoma exhibited lower levels of copper in ceruloplasmin (-22%) and iron in tranferrin (-33%) than healthy animals[19]. Indeed, following treatment of dogs with doxorubicin and l-asparaginase, plasma concentration of copper and iron reduced even more, -18% and -13%, respectively, what favors the hypothesis of chemotherapy leading to changes in ceruloplasmin levels. As dogs in this study were assigned to protocols without doxorubicin, the influence of other chemotherapies on copper metabolism for justifying ceruloplasmin decreasing should be further evaluated. Considering the hepatic synthesis of Cp, dogs with lymphoma showed changes in the serum activity of liver enzymes at diagnosis, which decreased with the beginning of treatment, but at no time during treatment showed other signs such as low albumin levels or jaundice to indicate greater impairment liver and could be justify the low levels of ceruloplasmin.

Finally, previous studies failed to identify an effect of chemotherapy itself on C-reactive protein and serum amyloid A concentrations in healthy dogs[12,13]. Somehow, this fact favors the possibility of ceruloplasmin be influenced indirectly by plasma levels of copper, as the mentioned proteins do not contain copper in their molecular structures.

In conclusion, ceruloplasmin levels remain unchanged in dogs with multicentric lymphoma at the disease diagnosis, and chemotherapy induces a decrease in ceruloplasmin levels in both healthy animals and in animals with lymphoma. The lack of induction by lymphoma and the influence by chemotherapy make ceruloplasmin an inappropriate acute phase protein for the monitoring of canine lymphoma.

Acknowledgment

This study was supported by FAPESP (Fundação de Ampar áà Pesquisa do Estado de São Paulo). The authors thank Marly Elizabeth Ferreira de Castro for assistance in performing the assays.

References

1. ECKERSALL, P. D. Acute phase proteins as markers of infection and inflammation: monitoring animal health, animal welfare and food safety. **Irish Veterinary Journal,** v. 53, p. 307-311, 2000.

2. CERÓN, J. J.; ECKERSALL, P. D.; MARTINEZ-SUBIELA, S. Acute phase proteins in dogs and cats: current knowledge and future perspectives. **Veterinary Clinical Pathology**, v. 34, n. 2, p. 85-99, 2005.

3. TECLES, F.; SPIRANELLI, E.; BONFANTI, U.; CERÓN, J. J.; PALTRINIERI, S. Preliminary studies of serum acute-phase protein concentrations in hematologic and neoplastic diseases of dogs. **Journal of Veterinary Internal Medicine**, v. 19, n. 6, p. 865-870, 2005.

4. MARTINEZ-SUBIELA, S.; TECLES, F.; PARRA, M. D.; CERÓN, J. J. Proteínas de fase aguda: conceptos básicos y principales aplicaciones clinicas en medicina veterinaria. **Annais Veterinaria Murcia,** v. 17, p. 99-116, 2001.

5. VAIL, D. M.; YOUNG, K. M. Hematopoietic tumors. In: WITHROW, S. J.; VAIL, D. M. **Small animal clinical oncology**. 4. ed. Missouri: Saunders Elsevier, 2007. p. 699-733.

6. MADEWELL, B. R. Diagnosis, assessment of prognosis and treatment of dogs with lymphoma: the sentinel changes (1973-1999). **Journal of Veterinary Internal Medicine**, v. 13, p. 393-394, 1999.

7. MYERS, N. C.; MOORE, A.; RAND, W. M.; GLIATTO, J.; COTTER, S. M. Evaluation of a multidrug chemotherapy protocol (ACOPAII) in dogs with lymphoma. **Journal of Veterinary Medicine**, v. 1, p. 333-339, 1997.

8. RASSNICK, K. M.; MAULDIN, G. E.; Al-SARRAF, R.; MAULDIN, G. N.; MOORE, A. S.; MOONEY, S. C. MOPP

chemotherapy for treatment of resistant lymphoma in dogs. A retrospective study of 117 cases (1989-2000). **Journal of Veterinary Internal Medicine**, v. 16, n. 5, p. 576-580, 2002.

9. CHILD, J. A.; SPATI, B.; ILLINGWORTH, S.; BARNARD, D.; CORBETT, S.; SIMMONS, A. V.; STONE, J.; WORTHY, T. S.; COOPER, E. H. Serum β-2 microglobulin and C-reactive protein in the monitoring of lymphoma**. Cancer**, v. 45, n. 2, p. 318-326, 1980.

10. GANZ, P. A.; SHELL, W. E.; TOKES, Z. A. Elevation of a radioimmunoassay for alpha 1 acid glycoprotein to monitor therapy of cancer patients**. Journal of the National Cancer Institute**, v. 71, p. 25-30, 1983.

11. LEGOUFFE, E.; RODRIGUEZ, C.; PICOT, M. C.; RICHARD, B.; KLEIN, B.; ROSSI, J. F.; COMMES, T. C-reactive protein serum level is a valuable and simple prognostic marker in non-Hodgkin lymphoma. **Leukemia Lymphoma**, v. 31, n.3/4, p. 351-357, 1998.

12. MERLO, A.; REZENDE, B. C. G.; FRANCHINI, M. L.; SIMÕES, D. M.; LUCAS, S. R. Serum C-reactive protein concentrations in dogs with multicentric lymphoma undergoing chemotherapy. **Journal of the American Veterinary Medical Association**, v. 230, n. 4, p. 522-526, 2007.

13. MERLO, A.; REZENDE, B. C. G.; FRANCHINI, M. L.; MONTEIRO, P. R.; LUCAS, S. R. R. Serum amyloid A is not a marker for relapse of multicentric lymphoma in dogs. **Veterinary Clinical Pathology,** v. 37, n. 1, p. 79-85, 2008.

14. SCHONSINSKY, K. H.; LEHMANN, H. P.; BEELER, M. F. Measurement of ceruloplasmin from its oxidase activity in serum by use of o-dianisidina dihidrochloride. **Clinical Chemistry**, v. 20, n. 12, p. 1556-1563, 1974.

15. MISCHKE, R.; WATERSTON, L. M.; ECKERSALL, P. D. Changes in C-reactive protein and haptoglobin in dogs with lymphatic neoplasia. **The Veterinary Journal**, v. 174, n. 1, p. 188-192, 2007.

16. HAHN, K. A.; FREEMAN, K. P.; BARNHILL, M. A.; STEPHEN, E. L. Serum α1-acid glycoprotein concentrations before and after relapse in dogs with lymphoma treated with doxorubicin. **Journal of the American Veterinary Medical Association**, v. 214, n. 7, p. 1023-1025, 1999.

17. SOLTER, P. F.; HOFFMANN, W. E.; HUNGERFORD, L.; SIEGEL, J. P.; STDENIS, S. H.; DORNER, J. L. Haptoglobin and ceruloplasmin as determinants of inflammation in dogs. **American Journal of Veterinary Research**, v. 52, n. 10, p. 1738-1742, 1991.

18. CERÓN, J. J.; MARTINEZ-SUBIELA, S.; OHNO, K.; CALDIN, M. A seven-point plan for acute phase protein interpretation in companion animals. **The Veterinary Journal**, v. 177, n. 1, p. 6-7, 2008.

19. GILLE, L.; KLEITER, M.; WILLMANN, M.; NOHL, H. Paramagnetic species in the plasma of dogs with lymphoma prior to and after treatment with doxorubicin – an ESR study. **Biochemical Pharmacology**, v. 64, n. 12, p. 1737-1744, 2002.